DEVELOPMENT OF THE ANGOUMOIS GRAIN MOTH, SITOTROGA CEREALELLA, (OLIV.), IN PELLETS OF VARIED COMPOSITIONS OF WHEAT GERM, BRAN AND ENDOSPERM UNDER CONTROLLED HUMIDITIES AND TEMPERATURE.

by

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INTRODUCTION

The Angoumois grain moth, Sitotroga cerealella, (Oliv.) is a destructive grain pest occurring in the United States and other parts of the world. It is most important in the southeastern part of the country causing great damage to corn in cribs and destroying ripening grain, especially wheat, in the field.

The insect received its name from the Province of Angoumois, France. A paper submitted by Duhamel and Tillet in 1762 entitled "History of an Insect which Devours the Grain of Angoumois" was the first detailed study of this insect.

The buff-colored adult is approximately one-fourth inch long and is commonly the only stage observed, but eggs and larvae may be seen occassionally by close observation. Eggs, about 0.6 millimeters long, are laid among kernels of grain. Larvae and pupae live entirely inside the seeds. Female moths may lay over 300 whitish eggs which turn reddish as they age. They may be laid singly or in clusters of 20 or more. In the laboratory they hatch in 4 to 6 days. In the field they may take up to four weeks due to the variation of environmental conditions. The newly-hatched caterpillars often build an external cocoon to gain leverage to burrow into the kernel where they feed upon the various parts, thus receiving their nutritional requirements. When fully grown, the larva prepares an exit tunnel leaving only a thin transparent "window" on the seed coat so the adult may emerge. Then it spins a thin, silken cocoon in the tunnel where it changes to a pupa. Under optimum conditions, the adult emerges 7 to 9 days later. The larval-pupal period usually lasts 35 to 40 days under these conditions. In Kansas there are likely to be two generations per year in stored grain.

Other studies have revealed considerable variability in the lengths of larval-pupal periods of these insects, even when reared under the same conditions. Such variability is undesirable in experimental studies using this insect. This study was undertaken to determine whether the life cycles of the Angoumois grain moth would develop more uniformly in pellets. Pellets were chosen as a physical medium because of their resemblance to the whole kernel in hardness and size, and because their physical and nutritional composition could be controlled. Uniformity in the life cycles would aid future experimental studies of this insect. Causes of variability in wheat are suspected to be nutritional, therefore the pellets were made of various compositions of bran, endosperm and germ, then placed in three different relative humidities.

Moisture content is recognized as one of the most important factors in stored grain insect nutrition. Relative humidities of 30%, 60% and 80% were chosen. Emergence of adults occurred only from media at 60% relative humidity although at 30% relative humidity some development was observed in the purified endosperm, 80% bran and ground whole wheat. Measurements of emergence weights, lengths, longevity of adults, larval-pupal time, number of instars, and weights after death were recorded.

REVIEW OF LITERATURE

The first reference to the Angoumois grain moth, Sitotroga cerealella, (Oliv.) was Reaumur's "Memories pour Servir a l'Histoire des Insects" (1736). He described but did not name the moth. Duhamel and Tillet (1762) reported on the first comprehensive study of the biology, ecology and control of this insect in the Province of Angoumois, France. Simmons and Ellington (1924,

1925, 1927, 1932, 1933) reported detailed studies of the Angoumois grain moth. Prior to the studies of Simmons and Ellington, the main sources of information were Reaumur (1736), Duhamel and Tillet (1762), King (1920), and Candura (1926). Simmons and Ellington (1932) completely summarized the literature up to that time.

The Angoumois grain moth has a wide variety of hosts including all the common grains. Warren (1954) studied the biology and behavior of the insect in 29 different corn hybrids and 22 sorghum varieties at different moisture levels.

Under certain conditions of moisture and temperature the larval-pupal period ranges from 30 to 42 days (Simmons and Ellington, 1933). Desphande (1929) observed that in rearing the moths from rice, under normal temperature and humidity, the larval-pupal period was 27 to 30 days. The actual larval developmental period, when reared on fine wheat flour, averaged 24 days (Crombie, 1943). Because moisture, temperature and rearing media influence the larval period, only generalizations can be made. Koone (1952) studied the life cycle in corn including incubation of eggs, larval-pupal period, fecundity, oviposition and development.

The Angoumois grain moth ranks second in resistance to drying of the grain (Farra, 1942). It was observed that when grain is at the higher moisture contents (up to a point) it is more favorable for insect activity. At lower humidities the greater part of the water formed in the body is from oxidation of food so more food is eaten (Fraenkel and Blewett, 1944).

Moore (1950) studied the effects of many substances of nutritional significance on various species of stored grain insects. Peters, Zuber and Fergason (1960) placed the Angoumois grain moths on corn with a high amylose content

and observed a slower life cycle. Fraenkel and Blewett (1943) reported on carbohydrates and sterol requirements of stored products insects. Basic work with vitamins was accomplished by Barton and Wright (1941) and Fraenkel and Blewett (1943). Nutritional studies of genetically different strains of insects was completed by Hinton and Dunlap (1956).

Factors of great importance relating to homogeneous mixtures of the rearing media, stickiness and particle size were stressed by Fraenkel and Blewett (1943) and Frobrich (1953). Kernel hardness was tested by Katz, Cardwell, Collins and Hostetter (1959, 1961).

Specific work relating to the nutritional requirements of the Angoumois grain moth is sparse in the literature.

Additional references are cited in this thesis where similar studies relate.

MATERIALS, METHODS AND SOME GENERAL TECHNIQUES

Source of Insects

Angoumois grain moths used in this experiment were taken from stock cultures maintained since August, 1960 at the Department of Entomology, Kansas State University.

Rearing Facilities of Stock Cultures

The stock cultures of Angoumois grain moths were reared in a Department of Entomology rearing room. The temperature was regulated at 80°F ± 2°F and the relative humidity at 65% ± 3%. They were reared in quart, wide-mouth mason jars, each containing approximately 300 grams of wheat and covered with a cap fitted with 60-mesh wire screen. Covering the caps were kelthane-treated

cloths to prevent mite infestation. Shelves on which the jars were placed were also covered with kelthane-treated cloth. Newly-emerged adults from a 40-day old culture were placed with wheat having 12.5% to 13.5% moisture content. By repeating this weekly, stock cultures were maintained.

Collection of Adults

Rearing jars containing emerged adults were opened inside a collection cage. Using a Cenco-Hyvac Vacuum Pump, the insects were aspirated into a 1" x 4" plastic vial, then transferred where desired.

Collection of Eggs

Adults were placed in $2\frac{1}{2}$" x 4" oviposition jars with metal caps (Plate I). The oviposition jars were modifications (Mills, 1965) of a type devised by Ellington (1930).

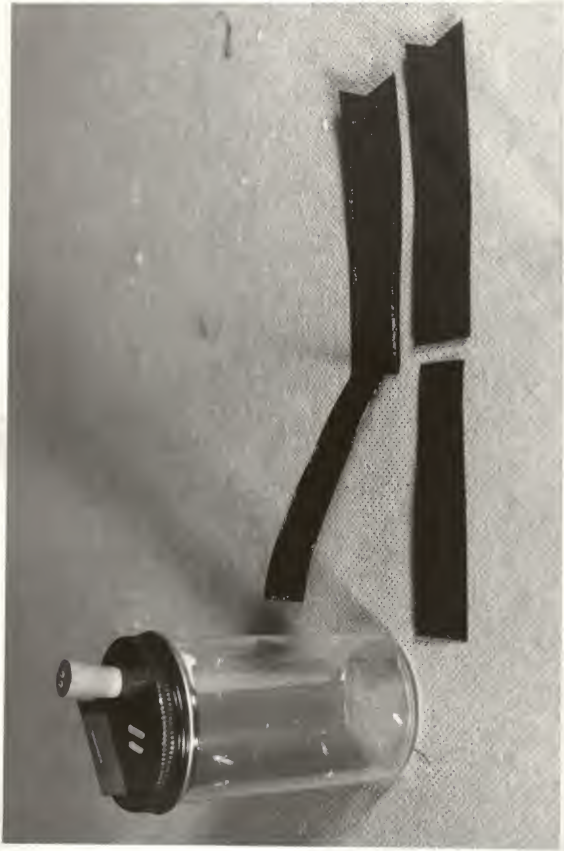
Female Angoumois grain moths will oviposit between strips of paper. Use of black construction paper provides a contrasting background for the white or reddish eggs. The black paper is cut into strips measuring $4\frac{1}{2}$" x $1\frac{1}{2}$" and $4\frac{1}{4}$" x $1\frac{1}{4}$". One strip is placed on the other and stapled at one end. A small piece of double sticky, clear tape is placed between the strips to hold them together. A "V" notch was cut into the longer strip at the end which rested on the bottom of the jar to give the moths more room for movement and to lessen the chance of injury when strips were inserted.

Slits were cut in the metal tops of the oviposition jars approximately $1\frac{1}{2}$" in length. The strips were inserted and removed through the slit in the lid without having to remove it. The double strip of black paper fitted snugly enough to prevent escape of moths. This apparatus facilitates collecting of the eggs without loss of moths.

EXPLANATION OF PLATE I

Oviposition jar and oviposition strips. The bottom two strips were stapled together and inserted through a slit in the oviposition jar cap. The top two strips, stapled together, were removed from an oviposition jar containing adult Angoumois grain moths. Clusters of eggs are noted along the edge of the strip. Note moth on side of jar.

PLATE I



Eggs were laid by the female moth inserting her abdomen between the paper strips approximately 1/8" from the edge. The strips were removed and pieces of the paper with adhering eggs were cut off and placed in an empty vial until hatching.

Gathering of the Larvae

The vials were placed in the rearing room. The eggs hatched in 5 to 6 days. For infesting kernels and pellets the larvae used were between 0 to 24 hours old. Newly-hatched larvae were picked up with a fine camel hair brush and transferred individually to the isolated kernel or pellet in a gelatin capsule.

Media

Source. The Hard Red Winter Wheat culture media used in this experiment were obtained from the Kansas State University Milling Department. The wheat had previously been separated into various compositions as determined by the Milling Department (Table 1).

Table 1. Chemical analysis of culture media.

	a/60% Germ	Purified b/Endosperm	Purified c/Bran	d/80% Bran*	Ground Whole Wheat and Whole Kernel**
Protein	29.30%	10.49%	17.16%	12.77%	11.30%
Ether Extract (Fat)	11.87%	00.86%	4.26%	1.76%	2.20%
Crude Fiber	2.12%	00.29%	9.46%	0.32%	
Ash	4.16%	00.40%	4.97%	0.86%	2.00%
Nitrogen Free Extract	37.34%	73.02%	54.32%	68.83%	66.40%
Carbohydrates	39.46%	73.31%	63.78%	69.15%	

a/Particles of germ with bran and endosperm mixed, b/Particles of endosperm quite free from bran, c/Seed coat with a small amount of endosperm and germ adhering to it, d/Coarse particles of bran mixed with some endosperm.

*The first four columns of percentages were determined by the Department of Biochemistry, Kansas State University.

**The percentages for whole wheat were taken from Maynard and Loosli (1962). These figures are for a different variety of hard red winter wheat, therefore, are close approximations to the variety used in this experiment. Whole kernels of variety used were not analyzed.

Preparation of Pellets. Various meals were ground using a Wiley Mill with a 40-mesh screen. Following grinding, the materials were moistened, then each was separately pelleted in a Thomas pellet press (Plate II). The pellets were placed carefully to dry in an open dish.

EXPLANATION OF PLATE II

Thomas pellet press used for making pellets.

PLATE II

Conditioning of Pellets. The pellets were sufficiently dry in 48 hours to be individually placed in gelatin capsules (Plate III), then placed in a battery jar with the desired relative humidity for a period of two weeks. During this period the moisture content of the pellets equilibrated with the relative humidity of the chamber (Table 2).

Table 2. Moisture content of pelleted media and whole kernels.*

Description	Percent of Moisture of Pellets at 30% Relative Humidity	- 60% Relative Humidity
60% Germ Pellets	11.6	12.8
Purified Endosperm Pellets	10.0	12.4
Purified Bran Pellets	10.1	13.1
Whole Kernel	10.5	12.8
80% Bran Pellets	10.1	12.4
Ground Whole Wheat Pellets	10.8	12.7

*Air oven moisture determination completed by author using standards set up by the Am. Assoc. Cereal Chem. (1962).

Weight Variation of Gelatin Capsules. A test was set up to determine the variance of moisture being absorbed by the gelatin capsules from day to day. It appeared that the weight changes of the different capsules varied consistently and could be compared to one another. These capsules were kept under constant relative humidity of 60% and a temperature of 80°F. While being weighed they were removed from this atmosphere for fifteen minutes (Table 3).

Housing for Pellets. Trays each made from a sheet of plastic and bordered with balsa wood were constructed to hold the pellets and unpelleted meals (Plates III, IV). A tiered structure also made of balsa wood was

EXPLANATION OF PLATE III.

Tray of gelatin capsules containing single pellets of various compositions.

PLATE III

Table 3. Determination of weight (mg) variation of empty gelatin capsules at 60% relative humidity and 80°F.

Days	A	B	C	D	E	F	G	H	I	J
0	134.65	133.65	130.20	129.60	128.95	129.60	127.10	124.50	118.25	122.80
1	127.10	136.00	132.40	132.35	131.30	132.00	129.70	126.80	120.65	125.10
2	138.00	136.85	133.15	133.00	132.15	132.75	130.55	127.80	121.00	125.95
3	137.60	136.55	132.90	132.65	121.80	132.30	130.30	127.00	121.00	125.60
4	137.00	136.00	132.45	132.20	131.50	132.15	129.80	126.90	120.70	125.00
5	137.35	136.10	132.55	132.15	131.40	132.25	130.00	127.00	120.50	125.15
6	137.20	135.80	132.65	132.00	137.25	132.00	129.65	126.55	120.50	125.15
7	136.00	135.85	132.45	132.00	131.00	132.90	129.90	126.55	120.40	125.15
8	137.20	136.10	132.60	132.40	131.55	132.15	130.00	126.80	120.65	125.15
9	137.20	136.00	132.60	132.40	131.55	132.15	129.85	126.80	120.65	125.15

EXPLANATION OF PLATE IV

Tray containing wheat meals of various compositions.

Left to right - upper row
whole kernels
purified bran
80% bran

Left to right - lower row
purified endosperm
60% germ
ground whole wheat

PLATE IV

constructed so that X-ray radiographs could be made of the pellets and kernels without removing them from the trays.

The tiered structures holding the trays were placed on glass platforms. These platforms were supported above the sulfuric acid solutions by four glass tubes.

The battery jars, sulfuric acid solutions, hygrometer, glass platforms and tiered structure with trays are shown in Plate V.

Attaining Desired Relative Humidity in Battery Jars

Three 5-gallon battery jars were used as containers in the experiment to make the desired relative humidity. The rearing room provided nearly constant temperature.

Constant relative humidities can be maintained in closed containers by the use of definite concentrations of sulfuric acid solutions (Solomon, 1951). Solutions of sulfuric acid (H_2SO_4) and water were premixed in pyrex glass beakers to prevent breakage of the battery jars due to heat produced during mixing. After cooling to room temperature the solutions were transferred to the battery jars. The sulfuric acid solutions used to produce the desired relative humidities were prepared according to Table 4.

Atmospheric relative humidity in each sealed jar was measured with an El-Tronics Hygrometer. Repeated weekly checks were made of each jar to insure maintainance of the proper relative humidity.

Determination of Instars. As the insect passes through all stadia within the kernel (or pellet), the cast larval head capsules remain inside. By careful work the mandibles may be found among the frass and debris inside the pellet. The pellet was placed in a depression of a porcelain spot plate and one or two drops of water were carefully placed on the pellet to soften it

EXPLANATION OF PLATE V

Battery jar used to provide desired relative humidity which was measured by an El-Tronics hygrometer. Approximately 2" of the appropriate concentration of sulfuric acid is in the bottom of the jar. Tiered balsa wood structures with trays containing the pellets are set on a glass platform supported over the acid by four glass tubes.

PLATE V

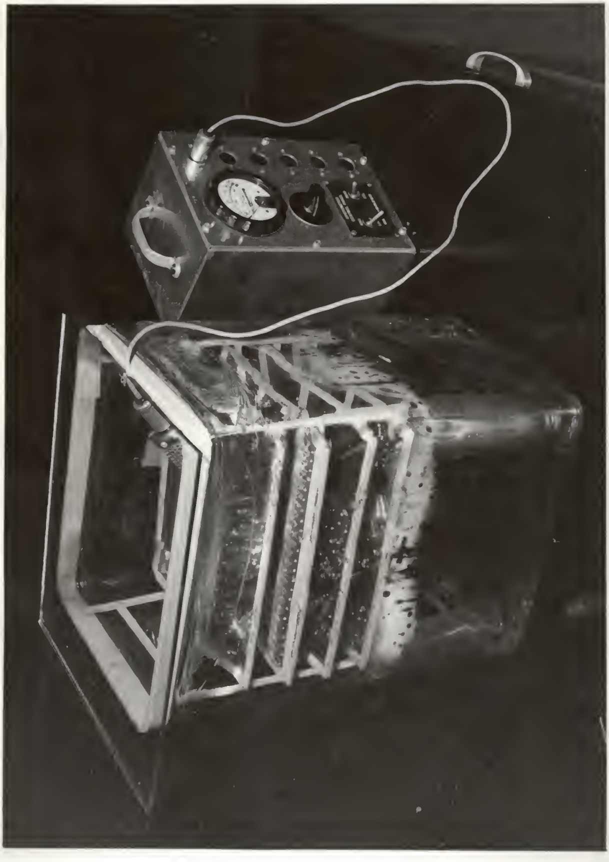


Table 4. Sulphuric acid solutions for control of desired atmospheric humidity (from Solomon, 1951).

R.H.% at 25°C.	Wt.% (g. H_2SO_4 per 100 g. solution.)	Density (g./ml.) at 20°C.
100	0	0.998
95	11.02	1.073
90	17.91	1.123
85	22.88	1.162
*80	26.79	1.193
75	30.14	1.219
70	33.09	1.245
65	35.80	1.267
*60	38.35	1.289
55	40.75	1.309
50	43.10	1.330
45	45.41	1.351
40	47.71	1.373
35	50.04	1.396
*30	52.45	1.419
25	55.01	1.445
20	57.76	1.474
15	60.80	1.507
10	64.45	1.546
5	69.44	1.604

*Relative humidities used in this experiment.

for dissection. Under binocular dissecting microscope fine needles made from insect pins were used to separate the mandibles from the frass and media. When a mandible was found, it was transferred to mineral oil for easy viewing and to avoid loss. The smaller mandibles were not always found because of their possible destruction by extensive feeding of the later instars or because of difficulty of seeing them among the debris of the pellet. By placing the mandibles found side by side, the different sizes could be determined. The number of instars was equal to the number of different sizes of mandibles (Plate VI).

Special Equipment

General Electric Grain Inspection X-Ray Unit. Radiographs were used to show rate of development and percent infestation in the pellets. X-ray radiographs were made with Eastman Kodak Type M Industrial X-ray film. Additional equipment necessary to prepare radiographs was supplied with the use of the X-ray machine.

Additional Equipment

A Mettler Type H-16 balance was used to weigh the insects and capsules. A binocular dissecting scope with calibrated ocular micrometer was used for taking body length measurements. A converted Barcol Impressor was used as a pellet hardness tester (R. Katz, et al., 1959).

Use of the X-ray Machine in the Study of Infestation

The use of the X-ray machine has proved itself indispensible in the study of insects that internally infest cereal grains. The insect developmental stage and instar often can be determined by observation of radiographs. Determination of the percent of infestation was made in this way.

EXPLANATION OF PLATE VI

Different sizes of mandibles of Angoumois grain moth used to determine the number of instars. Photograph was taken under mineral oil on a porcelain spot plate. Some of the mandibles are still attached to the head capsules.

PLATE VI

Preparatory tests showed that the best radiographs of pellets were made with X-ray settings of 27 kilovolts and 2.3 milliamperes for $2\frac{1}{2}$ minutes. Time was allowed for penetration of the plastic sheet in the bottom of the trays and gelatin capsules.

Standard developing procedures were used.

Photographs

Photographs were taken with an Exacta 35 millimeter camera using Kodak Plus X film. A Bausch and Lomb microscope lamp was used to illuminate the objects photographed through the microscope. The microscope with a 1X objective and a 15X ocular was used for photomicrographs.

RESULTS AND DISCUSSION

General Description of Pellet-Reared Insects.

The pellet-reared adult Angoumois grain moth ranged between 5 and 7.2 millimeters in length. This measurement was made from the tip of the head to the distal tip of the wings when horizontal along the back. The larval-pupal period ranged from 32 to 124 days dependent upon the rearing media. Adult weight averaged 1 to 3 milligrams more when reared in pellets than when reared in whole kernels. Normally, the adult Angoumois grain moth does not feed after emergence, therefore, it must use body-stored food and water. This was shown in a study of weight loss of adult insects from time of emergence from whole wheat kernels until four days after death. There was a definite tendency of the heavier ones to live longer (Table 5a, 5b). Although daily weights were not taken, pellet-reared adults exhibited comparable weight losses from emergence to four days after death.

Moths reared in pellets showed no abnormalities in general appearance, color, or activity.

Thirty-Percent Relative Humidity Group

Meals and Pellets. Growth and development of the Angoumois grain moth in 30% relative humidity were apparently impaired by low moisture content of the pellets (Table 2). No emergence of adults occurred. Early larval growth in the pellets was revealed by radiographs (Plate VII). No development of insects was observed in the meals (Plate VIII). Angoumois grain moth larvae were able to survive a short time in the moisture deficient environment probably due to the free water they carried in their bodies plus moisture obtained from unbound water in the pellet, and water obtained from metabolism of car-

Table 5a. Daily loss of weight of Angoumois grain moths* from 0-24 hours after emergence to four days after death. Insects reared in whole wheat kernels.

(weight in mg.)

	Females									
No. Days	1	2	3	4	5	6	7	8	9	10
0-24	2.35	4.05	3.80	5.55	3.75	4.60	3.55	2.95	4.45	3.60
1	*** --	--	--	--	--	--	--	--	--	--
2	1.60	3.30	2.75	4.00	2.70	3.90	3.00	1.70	3.35	2.60
3	1.60	3.15	2.70	4.10**	2.50	3.55	2.90	2.10**	3.25	2.70**
4	1.50	2.90	2.65	4.00	1.95	3.45	2.45	1.65	3.25	2.55
5	1.50	2.75	2.40	4.00	1.55	3.30	2.45	1.50	3.00	2.45
6	1.25	2.85	1.85	3.95	1.75	3.25	2.45	1.45	3.00	2.25
7	1.25	2.75	1.65	3.55	1.75	2.85	2.55	1.45	2.75	2.15
8	****1.00	2.55	1.65	3.55	1.50	2.85	2.20	1.20	2.60	2.00
9	1.00	2.35	1.30	3.35	1.15	2.70	1.65	1.20	2.50	1.85
10	.60	2.20	1.10	3.10	1.10	2.40	1.60	1.20	2.50	1.85
11	.60	2.00	.90	3.00	.80	2.40	1.35	.85	2.50	1.80
12	.60	2.00	.90	2.50	.80	2.30	1.00	.75	2.40	1.75
13		1.80		2.50	.80	2.25	1.00	.75	2.30	1.70
14		1.40		2.25	.80	2.00	1.00	.75	2.10	1.45
15		1.40		2.00		1.90			1.90	1.15
16		1.40		1.80		1.40			1.50	1.00
17				1.80		1.40			1.20	1.00
18				1.60					1.20	1.00
19				1.30					1.20	
20				1.30						
21				1.30						

*Reared at 60% relative humidity and 80°F.

**Increase in weights cannot be explained unless by error in weighing.

***Due to faulty procedures the weights for the first day were discarded.

****The figures underlined represent the day the insect died.

Table 5b. Daily loss of weight of Angoumois grain moths* from 0-24 hours after emergence to four days after death. Insects reared in whole wheat kernels.

(weight in mg.)

	Males									
No. Days	1	2	3	4	5	6	7	8	9	10
0-24	2.30	1.95	2.10	2.50	1.65	2.20	2.60	1.65	2.00	2.25
1	*** --	--	--	--	--	--	--	--	--	--
2	1.65	1.40	1.65	2.15	2.15	1.80	1.95	1.35	1.40	1.74
3	2.05**	1.35	1.30	1.80	1.95	1.70	2.35**	1.85**	1.30	1.85**
4	1.50	1.00	1.65	1.60	1.90	1.30	1.65	1.15	1.25	1.50
5	1.50	1.00	1.40	1.40	1.60	1.30	1.25	.90	1.00	1.50
6	1.45	1.00	1.25	1.65	1.55	1.30	1.25	.90	1.25	1.50
7	1.10	.80	1.10	1.20	1.55	1.00	1.25	.70	.95	1.00
8	1.10	.80	1.10	1.20	1.15	1.00	1.05	.65	.55	.95
9	1.10	.45	1.00	1.10	1.10	.80	.80	.55	.55	.90
10	**** .60	.45	.50	.85	1.00	.50	.65	.55	.50	.60
11	.55	.45	.50	.70	.75	.50	.65		.50	.55
12	.55	.45	.50	.70	.75	.50				.55
13	.55		.50	.70	.75	.50				
14					.75					

*Reared at 60% relative humidity and 80°F.

**Increase in weights cannot be explained unless by error in weighing.

***Due to faulty procedures the weights for the first day were discarded.

****The figures underlined represent the day the insect died.

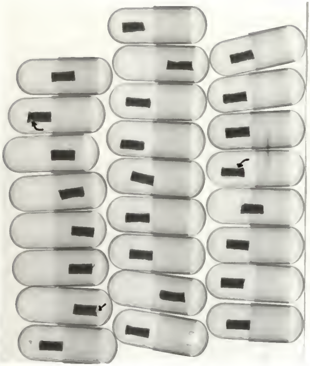
EXPLANATION OF PLATE VII.

Photographic prints made from X-ray radiographs of pellets of various compositions used to rear Angoumois grain moths in 30% relative humidity. Arrows indicate development of insects in some of the pellets. No emergence occurred.

A. 80% Bran Pellets
B. Purified Endosperm
C. Ground Whole Wheat

PLATE VII

A



B

C

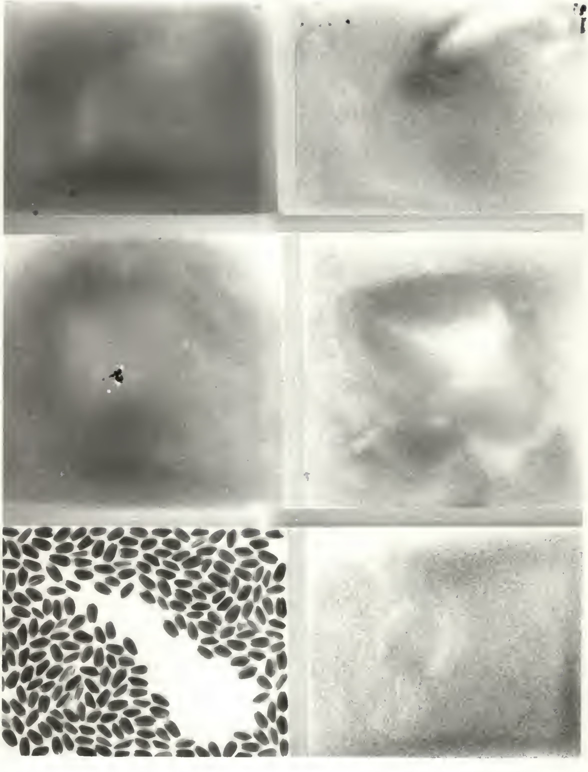
EXPLANATION OF PLATE VIII.

Photographic print made from X-ray radiograph of wheat meals and whole wheat kernels. No development appeared.

Left: upper - Purified bran
middle - 60% germ
lower - Whole wheat kernels

Right: upper - Ground whole wheat
middle - 80% bran
lower - Purified endosperm

PLATE VIII



bohydrates. This would seem logical according to the findings of Speicher (1931) and Fraenkel and Blewett (1944). Speicher, working with larvae of Ephestia kuehniella, found that they maintain constant percentage of free water independent of environment. Fraenkel and Blewett also observed that at low humidities the greater part of the water found in the insect body was from oxidation of food. At lower humidities more food was eaten.

Apparently insects in this study were not able to gain enough unbound water from the pellets or from the metabolism of carbohydrates to sustain life. Death of larvae in the 30% relative humidity was mainly attributed to low moisture content in the pellet. Other factors which possibly aided in the death of the larvae were shown by Frobrich (1953) who stressed particle size, hardness, stickiness and possibly selective feeding by the insect.

Hardness of pellets was tested with a converted Barcol Impressor. At lower humidities whole grains were only slightly harder than at higher humidities with some exceptions (Katz, et al., 1959). The same was found to be true with pellets in this study (Table 6).

Eighty Percent Relative Humidity Group

Meals and Pellets. Eleven days after pellets inside gelatin capsules were placed in 80% relative humidity to attain moisture content in equilibrium with the atmosphere, mold growth appeared on all the pellets except purified endosperm and whole kernels. The entire tray of meals was molded. Aspergillus mold, as determined by the Botany Department, Kansas State University, was the causative agent. After twenty days the purified endosperm and whole kernels molded. Purified endosperm took longer to mold probably because it was relatively clean from mold spores, coming from the interior of the kernel. The whole kernel delayed in molding probably due to the

Table 6. Hardness* of pellets as determined by a converted Barcol Impressor.

60% Relative Humidity	Number of Pellets										Average
Endosperm - end of pellet	26	28	30	47	35	37	38	41	35	31	34.8
side of pellet	57	43	50	55	53	51	52	49	50	54	46.8
Bran - end of pellet	Too soft to measure -										
side of pellet	- -										
80% Bran - end of pellet	25	30	34	30	28	29	30	34	36	33	30.9
side of pellet	40	43	40	42	39	40	43	40	42	41	41.0
60% Germ - end of pellet	4	3	6	8	4	3	6	2	5	6	4.7
side of pellet	9	8	12	14	7	7	14	8	9	13	10.1
Ground Whole Wheat - end of pellet	36	38	34	44	42	40	39	37	39	41	39.0
side of pellet	54	49	47	55	42	47	48	49	47	51	48.9
30% Relative Humidity											
Endosperm - end of pellet	32	42	40	53	40	41	39	37	40	38	40.2
side of pellet	58	56	50	48	52	50	49	48	53	50	51.4
Bran - end of pellet	Too soft to measure -										
side of pellet	- -										
80% Bran - end of pellet	25	36	24	38	25	30	28	33	31	29	29.9
side of pellet	50	56	45	49	52	50	49	56	57	49	51.3
60% Germ - end of pellet	10	12	14	9	8	10	9	11	10	8	10.1
side of pellet	10	6	24	17	12	15	18	12	14	13	14.1
Ground Whole Wheat - end of pellet	20	31	29	31	38	43	40	23	33	28	31.6
side of pellet	47	52	47	38	51	40	46	30	40	44	43.4

Whole Kernel (Wheat) tested between 35-45.

*numbers are relative - the higher the number, the harder the pellet (Katz, et al., 1959).

protection of its tough pericarp, especially the water impervious cuticle.

Gelatin capsules which housed the pellets appeared to absorb moisture quickly from the humid atmosphere. They became sticky and impossible to separate without becoming completely destroyed.

Due to Aspergillus mold growth in the high humidity, and also characteristic changes that made use of the gelatin capsule impossible, no infestation was induced and the pellets were discarded.

Sixty Percent Relative Humidity Group

Meals and Pellets. Moisture content (Table 2) of the pellets at 60% relative humidity was sufficient for insect development when proper nutrition was available. Higher humidity and moisture content is more favorable for stored grain insect development up to the point where molding of the grain begins. Chatterjee (1956) observed that the initial moisture content of the medium primarily determines the extent of damage. Among others that worked with moisture problems were Auber and Raymont (1944) and Cotton (1961).

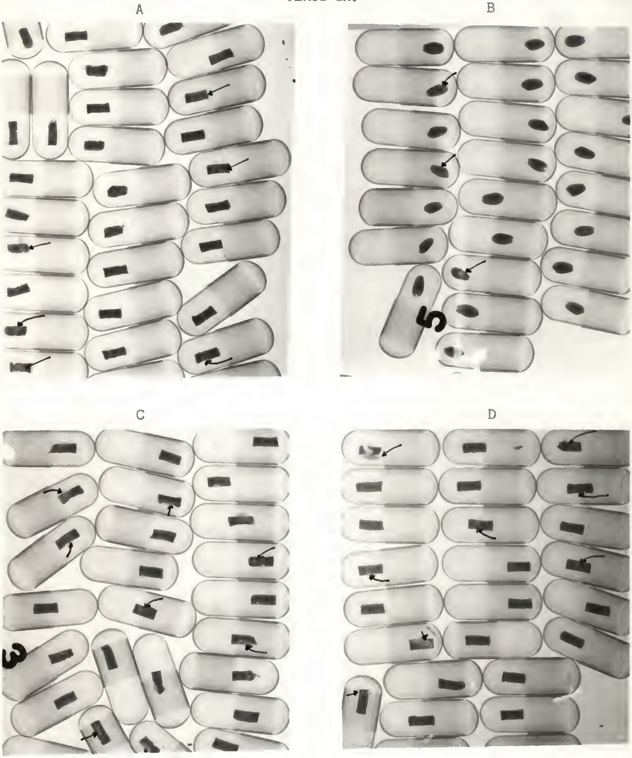
Purified Endosperm. Development was observed in X-ray radiographs after 30 days (Plate IX). Eighty percent of 50 pellets were found to be infested but only 44% of these insects emerged as adults. This could possibly be due to inadequate protein and vitamin in this medium. MacMasters, et al., (1964) reviewed previous research on structure and composition of the wheat kernel. The starchy endosperm contains in addition to starch, minute amounts of thiamin, niacin and pyridoxin but substantial amounts of protein, riboflavin, pantothenic acid. The outer endosperm has a higher proportion of protein than the inner. The aleurone layer, usually a single-cell layer immediately outside the starchy endosperm contains substantial amounts of vitamins. The aleurone layer is also richer in many of the amino acids.

EXPLANATION OF PLATE IX.

Photographic prints made from X-ray radiographs of pellets of various compositions used to rear Angoumois grain moths in 60% relative humidity. Arrows indicate development of insects in some of the pellets. Many of these insects emerged.

A. Ground Whole Wheat Pellets
B. Whole Wheat Kernels
C. Purified Endosperm Pellets
D. Eighty Percent Bran Pellets

PLATE IX.



This layer is removed with the bran during the milling process. Purified bran, which includes the aleurone and all the outer layers of the wheat kernel, and the germ are richer in sterols than the starchy endosperm. Vitamins of the B-group and sterols are necessary for growth of insects (Fraenkel and Blewett, 1943 a-b). Some of these essential nutrients are probably not found in optimum amounts in this medium, therefore, causing slower development and higher percent of mortality. Moths reared in pellets of purified endosperm weighed more than moths grown in whole wheat kernels, fewer moths reached maturity and development was much slower.

The larval-pupal period ranged from 60 to 124 days with an average of 98.04 days. This was the longest average developmental period of any of the groups. The longevity of adult males ranged from 7 to 17 days with an average of 11.3 days and the adult females ranged from 11 to 20 days with an average of 14.77 days. Ranges and averages of the larval-pupal period, longevity of adults, weight and length may be seen in Tables 7a, 7b.

Characteristic of insect growth in this medium were the conspicuous amounts of frass lying about the pellet (Plates X, XI).

The number of estimated instars ranged from 5 to 7. Usually, the longer larval-pupal periods produce a greater number of instars. Mills (1965) found seven instars in whole wheat kernels with shorter larval-pupal periods than observed in this study (Table 8).

No deviation from normal activity or color was observed.

Eighty Percent Bran Pellets. Infestation was observed by X-ray radiographs after 30 days (Plate IX). Sixty-six percent of the 50 bran pellets were found to be infested. Sixty percent of the insects in the 50 bran pellets emerged.

Table 7a. Larval-pupal periods and adult measurements of Angoumois grain moths from purified endosperm pellets at 60% relative humidity and 80°F.

GROUP I.

Sex	Larval-Pupal Period (Days)	Adult Measurements: Longevity in Days	Weight 0-24hr. (mg)	Length in mm.
M	60	13	4.20	6.633
M	64	8	2.40	5.529
M	90	10	2.70	5.820
F	90	12	4.15	5.820
M	94	13	3.30	5.820
M	100	8	2.80	5.626
F	100	13	4.00	6.111
F	106	16	4.00	5.626
M	106	17	4.00	5.820
F	106	20	4.60	5.626
F	113	14	5.20	5.529
Average of 11 Insects	93.50	13.00	3.81	5.815
Average of 6 Males	85.66	11.50	3.23	5.874
Average of 5 Females	103.00	15.00	4.49	5.742

Table 7b. Larval-pupal periods and adult measurements of Angoumois grain moths from purified endosperm pellets at 60% relative humidity and 80°F.

GROUP II.

Sex	Larval-Pupal Period (Days)	Adult Measurements: Longevity in Days	Weight 0-24hr. (mg)	Length in mm.
F	84	18	3.80	5.820
M	85	8	2.40	5.626
F	92	16	5.00	6.111
M	102	13	4.00	6.305
F	102	11	3.50	5.335
F	102	13	3.00	5.820
M	102	13	3.40	5.917
M	106	7	2.50	6.111
M	109	14	2.90	5.335
M	120	7	2.00	5.335
M	124	16	4.55	5.917
Average of 11 Insects	102.50	12.36	3.37	5.784
Average of 7 Males	106.85	11.14	3.11	5.792
Average of 4 Females	95.00	14.50	3.82	5.771
AVERAGE OF GROUP I AND II.				
Average of All Insects	98.04	12.72	3.56	5.799
Average of All Males	97.07	11.30	3.16	5.830
Average of All Females	99.44	14.77	4.25	5.755

EXPLANATION OF PLATE X.

Infested purified endosperm pellet in gelatin capsule with characteristic frass. The expelled frass was characteristic of the insects grown in this medium.

PLATE X

EXPLANATION OF PLATE XI

Pupal case and larval head capsule of an Angoumois grain moth, and frass in a completely destroyed pellet. The frass may be identified by the large white balls.

PLATE XI

Table 8. Number of individual Angoumois grain moth instars recorded from selected pellets of various compositions.

Media	Larval-Pupal Period (Days)	Instars Observed*	Instars Estimated**
Ground Whole Wheat	63	6	7
	65	6	7
	93	6	7
	113	7	9
Purified Endosperm	60	4	5
	94	5	6
	106	5	6
	124	6	7
80% Bran	32	4	4
	50	5	5
	63	6	7
	68	6	7
Whole Wheat Kernel (Mills, 1965)	32	4	
	35	5	
	47	6	
	75	7	

*Number of different sizes of cast mandibles actually found in pellets.

**By comparing sizes of mandibles found with those on a series of microscopic slides previously prepared by the Department of Entomology, Kansas State University, it was obvious that in some cases the smaller mandibles were not found.

With the exception of four insects that emerged in 32 to 36 days, larval-pupal periods were somewhat more uniform but considerably longer than in whole wheat kernels. The larval-pupal period ranged from 32 to 70 days with an average of 56.8 days (Table 9a, 9b).

The relatively uniform larval-pupal period, the higher percent of infestation and emergence, and the larger average size of the insects makes this medium appear to be the most satisfactory for the development of this insect. Possibly sufficient carbohydrates, more adequate protein, a low ash content, adequate moisture and the supplying of the necessary nutrients for normal growth hastened development. No definite amount of time may be alloted to the so called "normal" larval-pupal period; according to Crombie (1943) only generalizations can be made concerning the larval-pupal period due to the specific influences of moisture, temperature and rearing media.

The number of instars increased as the larval-pupal period increased. These ranged from 4 to 7 instars (Table 8). Normal color and activity was observed.

Ground Whole Wheat (Table 10). Development in this medium was observed in X-ray radiographs (Plate IX) 30 days after infestation with larvae 0-24 hours old. Twenty-four percent of 50 pellets became infested but only 14% of these insects emerged as adults. In preliminary testing with ground whole wheat, 56% of the pellets became infested while 48% of the insects emerged as adults. Both tests were at 60% relative humidity and 80°F. Causes of the differences were not discovered.

Possibly the difference in infestation and number of emerged adults as compared to the purified endosperm and 80% bran media was due to the slight increase in fat and ash content. Fat increase may be detrimental to insect development due to an inhibitory effect upon the openings and passages of

Table 9a. Larval-pupal periods and adult measurements of Angoumois grain moths from 80% bran pellets at 60% relative humidity and 80°F.

GROUP I

Sex	Larval-Pupal Period (Days)	Adult Measurements: Longevity in Days	Weight 0-24hr. (mg)	Length in mm.
M	32	7	3.03	6.402
F	36	18	3.23	6.633
F	49	10	3.43	6.208
M	51	9	2.75	6.153
M	52	11	3.25	5.820
F	57	17	3.56	6.014
M	59	11	3.00	6.014
F	59	11	3.40	6.305
F	62	15	5.95	6.305
F	63	13	5.40	7.178
M	64	16	5.15	6.305
F	64	20	4.75	5.626
F	64	8	3.95	6.014
M	67	3	3.89	6.402
M	70	11	3.15	5.820
Average of 15 Insects	56.60	12.00	3.86	6.203
Average of 7 Males	56.42	9.71	3.46	6.131
Average of 8 Females	56.75	14.00	4.20	6.285

Table 9b. Larval-pupal periods and adult measurements of Angoumois grain moths from 80% bran pellets at 60% relative humidity and 80°F.

GROUP II

Sex	Larval-Pupal Period (Days)	Adult Measurements: Longevity in Days	Weight 0-24hr. (mg)	Length in mm.
M	32	5	2.88	5.626
M	36	12	3.35	6.596
M	62	10	3.85	6.596
M	55	10	3.13	5.917
M	55	10	3.32	5.917
F	55	17	3.27	5.820
M	55	8	2.61	5.917
M	56	11	3.25	5.917
M	57	11	2.80	5.820
M	57	12	3.00	5.820
F	60	15	5.50	6.494
M	62	9	5.78	5.529
F	64	15	5.00	6.790
M	66	8	2.42	5.917
M	67	9	3.66	6.499
Average of 15 Insects	55.26	10.80	3.58	6.079
Average of 12 Males	54.16	9.58	3.34	6.002
Average of 3 Females	59.66	15.66	4.59	6.370
AVERAGE OF GROUP I AND II				
Average of All Insects	55.93	11.40	3.72	6.146
Average of All Males	55.00	9.63	3.39	6.051
Average of All Females	57.54	14.45	4.31	6.307

Table 10. Larval-pupal periods and adult measurements of Angoumois grain moths from ground whole wheat at 60% relative humidity and 80°F.

GROUP I

Sex	Larval-Pupal Period (Days)	Adult Measurements: Longevity in Days	Weight 0-24hr. (mg)	Length in mm.
M	63	13	1.95	6.197
M	65	10	2.95	5.432
F	84	13	3.35	6.013
M	86	10	3.90	5.917
M	93	9	3.10	5.820
Average of 5 Insects	78.00	11.00	2.85	5.875
Average of 4 Males	76.70	10.50	2.72	5.841
One Female	84.00	13.00	3.35	6.013

GROUP II

Sex	Larval-Pupal Period (Days)	Longevity in Days	Weight 0-24hr. (mg)	Length in mm.
F	90	12	2.20	5.305
M	113	12	3.15	5.917
Average of 2 Insects	101.50	12.00	2.67	5.611
One Male	113.00	12.00	3.15	5.917
One Female	90.00	12.00	2.20	5.305

AVERAGE OF GROUP I AND II

	Larval-Pupal Period (Days)	Longevity in Days	Weight 0-24hr. (mg)	Length in mm.
Average of All Insects	84.85	11.28	2.80	5.800
Average of All Males	85.00	10.80	2.81	5.856
Average of All Females	87.00	12.50	2.77	5.659

the respiratory system, causing them to become blocked to the penetration of air. Slight increase in the amount of ash might have had some effect on development.

The number of estimated instars ranged from 4 to 9 (Table 8). No deformities were observed and color and activity appeared to be normal.

Whole Wheat Kernels. Development in whole kernels was observed in X-ray radiographs after two weeks. Fourteen percent of the 50 kernels became infested and 12% of the moths emerged (Table 11). The low percent of infestation could be caused by the strain of hard red winter wheat and the quality of the hand-picked kernels used. Compared to randomly selected kernels, the hand-picked ones contained fewer cracks in the seed coat over the germ which provides easy entrance by the larvae into the kernel.

Determination of the number of instars of larvae in the whole kernels was not attempted by the author. Simmons and Ellington (1933) and Crombie (1943) found the number of instars to be four. DeCarvalho (1963) stated "The larvae which undergo diapause, in relatively large numbers, have one more ecdysis," resulting in five instars. Mills (1965) found from 4 to 7 instars and stated "more instars were associated with longer larval-pupal periods," but found no evidence of diapause (Table 8).

The color and activity of the moths appeared normal.

Purified Bran and 60% Germ Pellets. Development in these two media was not observed by either X-ray or microscope. The pellets contained adequate moisture for stored grain insect development. The lack of development may have been due to the relatively high fat and/or ash content in both of these media, or lack of certain undetermined nutrients.

Table 11. Larval-pupal periods and adult measurements of Angoumois grain moths from whole kernels at 60% relative humidity and 80°F.

Sex	Larval-Pupal Period (Days)	Adult Measurements: Longevity in Days	Adult Measurements: Weight 0-24hr. (mg)	Adult Measurements: Length in mm.
GROUP I				
M	38	19	3.25	5.982
M	43	9	3.41	5.917
M	49	17	3.28	6.111
M	52	4	1.90	5.820
Average of 4 Males	45.00	12.00	2.96	5.957
GROUP II				
M	44	13	2.42	5.626
M	58	4	2.14	5.626
Average of 2 Males	51.00	8.50	2.28	5.626
AVERAGE OF GROUP I AND II				
Average of All Males	47.33	11.00	2.73	5.847

SUMMARY

Angoumois grain moths, Sitotroga cerealella (Oliv.), were reared in various wheat media under constant conditions of 30, 60 and 80 percent relative humidities at 80°F. A variety of hard red winter wheat was used. The wheat was ground and separated into meals of different compositions at the Kansas State University Milling Department.

The meals were of the following compositions: ground whole wheat, purified endosperm, 80% bran, 60% germ and purified bran. Hand-picked whole wheat kernels were also used. The different media were then pelleted with a Thomas pellet press and each pellet placed in a gelatin capsule which in turn was placed in a chamber having the desired relative humidity until the moisture content of the pellet reached equilibrium. Each pellet was then infested with a single, 0-24 hour old larva and returned to the chamber.

This study was done to determine the variability of the life cycles of the Angoumois grain moth under different conditions. Determining a suitable medium and moisture to obtain a more uniform life cycle would aid in future experimental studies of this insect.

No emergence was recorded from the pellets and meals maintained in the 30% relative humidity probably due to the low moisture content. The larvae did, however, develop for a short period as evidenced by X-ray radiographs.

The 80% relative humidity produced a high moisture content in the pellets and Aspergillus mold made this media unfit for use in this experiment.

Growth and development in the 60% relative humidity apparently was dependent upon compositions of the various media (Table 1). There was complete development of insects in purified endosperm, 80% bran, ground whole wheat and whole kernels but no apparent development in purified bran and 60% germ (Table 12). The percent of infestation in each medium was: purified endosperm,

Table 12. Summary of results of Angoumois grain moth in 50 pellets of each of the various rearing media at 60% relative humidity and 80°F.

	Purified* Endosperm	80% Bran*	Ground* Whole Wheat	Whole* Kernels	Purified Bran*	60% Germ*
Percent of Infestation	80	66	24	14	0	0
Number of Emergents	22	30	7	6	0	0
Percent of Emergence	44	60	14	12	0	0
Larval-Pupal Period (days)	60-124	32-70	63-113	38-58	0	0
Average	98.04	55.93	84.85	51.00	0	0
Longevity of Adults (days)						
Males - Range	7-17	3-16	9-13	4-19	0	0
Average	11.30	9.63	10.50	11.00	0	0
Females - Range	11-20	8-20	12-13	0	0	0
Average	14.77	14.45	12.50	0	0	0
Weight of Adults (mg)						
Males - Range	2.00-4.55	2.42-5.78	1.95-3.15	1.90-3.41	0	0
Average	3.16	3.39	2.85	2.73	0	0
Females - Range	3.00-5.20	3.23-5.95	2.20-3.35	0	0	0
Average	4.25	4.31	2.77	0	0	0
Length of Adults (mm)						
Males - Range	5.33-6.62	5.52-6.59	5.43-6.19	5.62-6.11	0	0
Average	5.83	6.05	5.85	5.84	0	0
Females - Range	5.33-6.11	5.62-7.17	5.30-6.01	0	0	0
Average	5.75	6.30	5.65	0	0	0

*See descriptions of media, page 9.

80%; 80% bran, 66%; ground whole wheat, 24%; whole kernels, 14%. The percent of larvae placed with the media which completely developed and emerged as adult were: purified endosperm, 44%; 80% bran, 60%; ground whole wheat, 14%; and whole kernel, 12%.

The larval-pupal period varied most in purified endosperm, 60 to 124 days with an average of 98.04 days. With the exception of four insects which had larval-pupal periods between 32 to 36 days, there was less variability among those reared in 80% bran. However, the larval-pupal periods were longer than in whole kernels, ranging from 50 to 70 days with an average of 56.9 days.

The number of instars was determined for the insects reared in different media by counting cast larval mandibles left in the pellets (Table 8). The longer the larval-pupal period the more instars in the life cycle. The number of instars determined for insects reared in the various media ranged from: ground whole wheat, 7 to 9; purified endosperm, 5 to 7; 80% bran, 4 to 7; and whole wheat kernel, 4 to 7.

There was an attempt to rear insects in unpelleted meals of the same compositions and in the same environments as the pellets. This proved unsuccessful for all meals in the three relative humidities.

Angoumois grain moths can be reared in pellets. Pelleted eighty percent bran rearing medium proved best of all the media used in this study for eliminating variability in the larval-pupal period. Hardness of the pellet appeared to have no effect on development. Purified endosperm pellets produced the longest average larval-pupal period, 98.04 days. Thirty percent relative humidity was not a suitable atmosphere for Angoumois grain moth development, and 80% relative humidity caused spoilage of media. A relative humidity of

60% appeared adequate for insect development and differences among the insects reared in the various media was apparently due to variable nutrition.

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DEVELOPMENT OF THE ANGOUMOIS GRAIN MOTH, SITOTROGA CEREALELLA, (OLIV.), IN PELLETS OF VARIED COMPOSITIONS OF WHEAT GERM, BRAN AND ENDOSPERM UNDER CONTROLLED HUMIDITIES AND TEMPERATURE.

by

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ABSTRACT

This study was to determine whether or not the Angoumois grain moth, Sitotroga cerealella (Oliv.), would develop in pellets and if the normal variability of the length of life cycle of this insect could be reduced. The usual variability in wheat is undesirable in experimental studies of this insect. Pellets were chosen as physical media because of their resemblance to the whole kernel in hardness and size. Media in form of meals were also used. The advantage in using pellets is the ability to vary the nutritional composition.

This moth was studied under constant conditions of 30 and 60 percent relative humidities at 80°F. Because of mold growth on media, attempts to rear the insects at 80 percent relative humidity were unsuccessful. Hard red winter wheat culture medium was used. The wheat was ground and separated into meals of different compositions by the Kansas State University Milling Department.

The meals were of the following compositions: ground whole wheat, purified endosperm, 80% bran, 60% germ and purified bran. Hand-picked whole kernels were also used. The different meals were then pelleted in a Thomas pellet press and each pellet placed in a gelatin capsule which in turn was placed in a chamber having the desired relative humidity until the moisture content of the pellet reached equilibrium. Each pellet was then infested individually with a larva 0 to 24 hours old and returned to the chamber.

No emergence was recorded from the 30% relative humidity group, probably because of the low moisture content. Some larvae did, however, develop for a short period as evidenced by X-ray radiographs.

The 80% relative humidity produced a high moisture content in the pellets and Aspergillus mold made this media unfit for use in this experiment.

Since there was apparently ample moisture, variations in growth and development of insects in 60% relative humidity probably resulted from the different compositions of the various media. There was complete development of insects in purified endosperm, 80% bran and ground whole wheat and whole kernels, but no apparent development in purified bran and 60% germ.

The lengths of the larval-pupal periods, longevity, weights and lengths of adults, percents of infestation and emergence, and numbers of instars were determined.

Eighty percent bran, with the exception of four insects which had larval-pupal periods between 32 to 36 days, proved to produce the least variable larval-pupal period. However, the larval-pupal periods were longer than normally found in whole wheat, ranging from 50 to 70 days (excluding above four insects) with an average of 56.9 days.

There was an attempt to rear insects in unpelleted meals of the same compositions and in the same environments as the pellets. This proved unsuccessful for all meals in the three relative humidities.